Harmful Algae 48 (2015) 63–68

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Distribution and population dynamics of potential anatoxin-a-producing cyanobacteria in Lake Dianchi, China

Suqin Wang [a,b], Lin Zhu [a,b], Qi Li [c], Genbao Li [a,*], Lin Li [a], Lirong Song [a], Nanqin Gan [a,*]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Wuhan Foreign Language School, Wansongyuan Road 48, Wuhan 430022, PR China

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 7 July 2015
Accepted 7 July 2015
Available online 28 July 2015

Keywords:
Anatoxin-a
Aphanizomenon
Anabaena
Quantitative PCR
Lake Dianchi

ABSTRACT

The occurrence of cyanobacterial blooms is often accompanied by a variety of toxic secondary metabolites known as cyanotoxins. Anatoxin-a (ATX-a) is a highly toxic cyanobacterial neurotoxin synthesized by numerous species (e.g., *Aphanizomenon*, *Anabaena* and *Oscillatoria*) that has received much public attention. In this study, we used molecular methods (PCR and qPCR) to track the presence and dynamics of ATX-a-producing cyanobacteria, *Aphanizomenon* and *Anabaena* in Lake Dianchi, China based on the *anaC* and *cpc*BA-IGS genes over a 23-month period (from June 2010 to April 2012). Results revealed that *Aphanizomenon* was the major potential ATX-a producer in Lake Dianchi and that they were most abundant in early spring and least abundant in summer, coinciding with observed *Aphanizomenon* blooms. It was found that the proportion of ATX-a toxigenic cells was lower in the northern part of the lake (2.1%) than the middle (16.7%) and southern parts (19.2%). The information on the spatio-temporal distributions of ATX-a-producing cyanobacteria obtained in this study will help to build management strategies to improve water quality for public health.

1. Introduction

Cyanobacteria are very ancient phytoplanktons that live in almost all possible habitats: water, soil and rocks (Méjean et al., 2014). Some of the species can form large scale blooms in eutrophic freshwater around the world. Cyanobacteria produce a wide range of toxic compounds, including neurotoxins and hepatotoxins, which can have adverse effects on environmental and public health (Sivonen, 1996). Anatoxin-a (ATX-a), one of the main neurocyanotoxins produced by several genera of cyanobacteria, including *Anabaena*, *Aphanizomenon*, *Oscillatoria*, *Cylindrospermum*, *Planktothrix*, *Phormidium* and *Tychonema*, was first identified from an *Anabaena flos-aquae* strain in the 1970s (Osswald et al., 2007; Shams et al., 2015; Devlin et al., 1977). It exhibits extreme toxicity, killing mice within 2–5 min, and was initially known as "very fast death factor" (Devlin et al., 1977). ATX-a acts as a post-synaptic depolarizing agent that can disrupt normal signal transmission between neurons and muscles, leading animal to death via respiratory arrest (Aronstam and Witkop, 1981; Dar and Zinder, 1998). It has been responsible for the poisonings of many animals, including cows, dogs, mice and cattle (Osswald et al., 2007). ATX-a also adversely impacts aquatic plants and zooplankton (Mitrovic et al., 2004; Ha and Pflugmacher, 2013; Claska and Gilbert, 1998).

Various analytical methods has been developed for the detection of ATX-a, most reported methods are based on the High Performance Liquid Chromatography (HPLC) coupled with mass spectrometry (MS), or by Gas Chromatography coupled with MS (Namera et al., 2002; Himberg, 1989). These methods, however, may meet some difficulties when employed to a long period field survey. For ATX-a is unstable in nature conditions (Kaminski et al., 2013) and the analysis of numerous field samples can be very time-consuming. Therefore, a rapid on-site monitoring method is needed. Quantitative polymerase chain reaction (qPCR) has been successfully applied to the on-site survey of harmful cyanobacteria blooms based on the functional genes which involved in the biosynthesis of cyanotoxins (Baxa et al., 2010; Conradie and Barnard, 2012; Rinta-Kanto et al., 2005).

In recent years, the biosynthesis of anatoxins at genomic level has achieved significant progress (Méjean et al., 2014). The functional genes responsible for ATX-a biosynthesis were

* Corresponding authors. Tel.: +86 27 68780210; fax: +86 27 68780871.
E-mail addresses: ligb@ihb.ac.cn (G. Li), gannq@ihb.ac.cn (N. Gan).

http://dx.doi.org/10.1016/j.hal.2015.07.005
1568-9883/

successively identified in four strains, *Oscillatoria* sp. PCC 6506 (Méjean et al., 2009), *Anabaena* sp. 37 (Rantala-Ylinen et al., 2011), *Oscillatoria* sp. PCC 6407 and *Cylindrospermum stagnale* PCC 7417 (Shih et al., 2013). Since then, additional molecular methods have been developed to detect the *anaF* gene, which encodes for a polyketide synthase critical for ATX-a biosynthesis by *Aphanizomenon* (Ballot et al., 2010), *Oscillatoria* (Cadel-Six et al., 2009), and *Phormidium* (Wood et al., 2010), as well as the *anaC* gene that encodes for the AnaC adenylation domain responsible for the activation of proline (Rantala-Ylinen et al., 2011).

The genera *Aphanizomenon* and *Anabaena* are the most prevalent planktonic cyanobacteria, along with *Microcystis*, responsible for cyanobacteria blooms worldwide. Phylogenetic analyses of cyanobacteria are commonly based on sequence variation in the 16S rRNA gene. It cannot differentiate evolutionary relationships between the genera *Aphanizomenon* and *Anabaena* (Gugger et al., 2002), however. Similarly, *Aphanizomenon* and *Anabaena* strains could not be distinguished in a study that used both 16S rRNA and *rpoB* gene sequences (Rajaniemi et al., 2005). Recently, the phycocyanin intergenic spacer (*cpc*BA-IGS) was used successfully to distinguish *Dolichospermum flos-aquae* from *Aphanizomenon flos-aquae* and *Aphanizomenon gracile* in Taihu Lake (Wang et al., 2013).

In Lake Dianchi, China, cyanobacteria blooms occur throughout the year with *Aphanizomenon* blooms most common in cooler seasons and *Microcystis* blooms most common in warmer seasons (Liu et al., 2006). Over the past decades, *Microcystis* and its hepatotoxin have been extensively studied. But no information is available about dynamics of ATX-a-producing cyanobacteria like *Aphanizomenon* or *Anabaena*. Hence, the current study aims at revealing the population dynamics of ATX-a-producing cyanobacteria as well as its potential producers *Aphanizomenon* and *Anabaena* in Lake Dianchi over a 23-month investigation.

2. Materials and methods

2.1. Study site and sampling

Lake Dianchi is located in Yunnan Province, southwest China. Samples were collected monthly from June 2010 to April 2012 at a depth of 0.5 m. Sampling was carried out at three sites: a northern site (Station-D24, 24°57′39.17″ N, 102°38′45.40″ E), middle site (Station-D13, 24°49′48.00″ N, 102°42′47.00″ E), and a southern site (Station-D22, 24°41′58.00″ N, 102°39′53.00″ E) (Fig. 1). Duplicate 50 ml water samples were collected with two van Dorn bottles and then filtered through 0.22 μm-pore-size polycarbonate membrane filters (CA, China). Filters were stored at −20 °C for DNA extraction. Concentrations of total nitrogen (TN) and total phosphorus (TP) were carried out using standard methods (APHA, 1998). Water temperature and pH were determined on-site using a YSI probe (YSI 650A, USA) and the water transparency was measured with a Secchi disc.

2.2. Cyanobacterial cultures

Seventeen *Aphanizomenon* strains and five *Anabaena* strains isolated from Lake Dianchi were used for designing *Aphanizomenon*-specific primers as well as for anatoxin-a detecting (Table S1). These strains were single filaments and non-axenic. The taxonomic identification of these strains included the morphological analyses (Komárek, 2013) and further genetic characterization based on *cpc*BA-IGS and 16S rRNA genes. Besides, thirty strains (including thirteen *Aphanizomenon* spp., eight *Anabaena* spp. three *Oscillatoria* spp., two *Nostocs* spp., one *Planktothrix* sp. and three *Microcystis* spp.) were used to verify the specificity of the designed primers. Among all the strains, FACHB strains were obtained from Freshwater Algae Culture Collection of Institute of Hydrobiology (http://algae.ihb.ac.cn/English/). Other strains were from our laboratory. All strains were maintained at 20 ± 1 °C in BG11 medium, illuminated under a constant cool-fluorescent light with an intensity of 25 μmol photons s^{-1} m^{-2}, on a 12 h:12 h light/dark cycle.

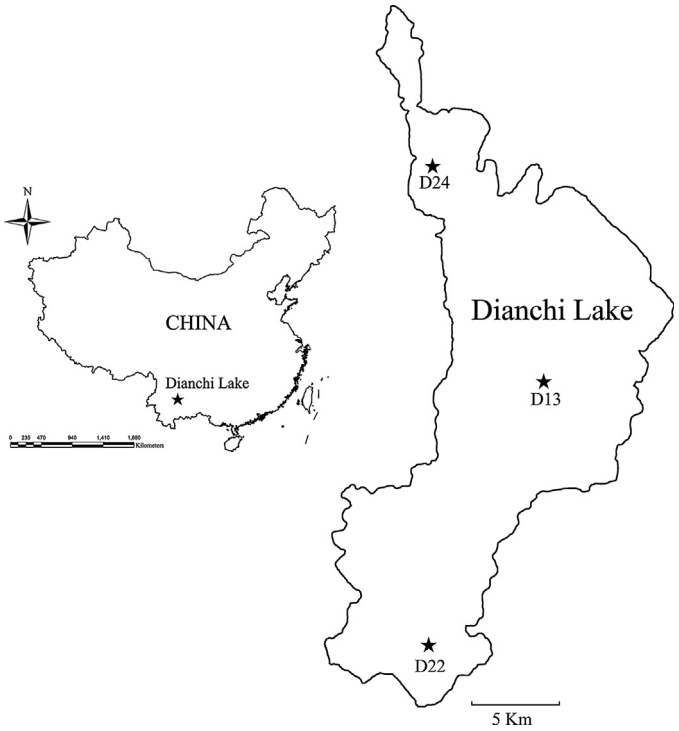

Fig. 1. Sampling sites in Lake Dianchi, southwest China (D24: 24°57′39.17″ N, 102°38′45.40″ E; D13: 24°49′48.00″ N, 102°42′47.00″ E; D22: 24°41′58.00″ N, 102°39′53.00″ E).

2.3. DNA extraction

Total genomic DNA was extracted from frozen filters and pure cultures with the EZNA Water DNA Kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. The filtration membranes were cut into small pieces with sterilized scissors and then lysed in lysis buffer using beating beads. DNA was extracted from the pellets using a HiBind DNA spin-column. DNA concentrations were measured with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

2.4. PCR amplification and sequencing of the anaC gene

To detect the presence of potential ATX-a cyanobacteria in field samples and pure cultures, genomic DNAs of field samples and pure cultures were initially examined by conventional PCR based on the *anaC* gene, published primer pair anxgen (861 bp) (Table 1) was used following the protocols in Rantala-Ylinen et al. (2011). PCR products were checked by electrophoresis on 1.2% (w/v) agarose gels and visualized with a Imaging System (UVP, USA). Samples that did not amplify the *anaC* gene were considered negative for ATX-a production. Positive DNA bands were purified using a PCR purification kit (BioerTech, China), then purified DNA were cloned into pMD18-T vector systems (Takara, Japan) and sequenced with the ABI 3730 Automated Sequencer (PerkinElmer Biosystems, USA).